

FACTORS CONTROLLING VARIATIONS IN THE RATE OF TRANSPIRATION.

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While investigating the relation of hairy leaf coverings to the resistance of leaves to water loss,* a number of experiments were performed which show the relative effects of several of the principal factors controlling transpiration and the rhythm of the transpiration curve in darkness in mullein and tobacco. Because these experiments were not directly related to the subject of hairy leaf coverings and transpiration they are presented separately in this paper.

HISTORICAL.

Brown and Escombe¹ in their researches on static diffusion of gases showed that the stomatal openings of a leaf act the same as a multiperforate diaphragm in allowing water vapor to pass out from the intercellular spaces of the leaf. They showed that, in view of the number and sizes of the stomata, only about one-sixth of the possible diffusion of water vapor from the leaf of a sunflower (*Helianthus* sp.) is ever attained under ordinary conditions. Lloyd² came to the same conclusion in his experiments with ocotillo (*Fouquieria splendens*) and showed that when the stomata are almost closed their diffusion capacity is much greater than is needed even to allow a maximum transpiration. He concludes that the movements of the guard cells tending to open and close are not sufficient to account for the variations of water loss, under those conditions. He says, however, that complete closure (if it ever does occur) would function in controlling transpiration by eliminating the evaporation from the internal surface of the leaf and reducing the water loss to that from the cuticular surface. Livingston and Estabrook³ found in a number of plants (*Funkia*, *Isatis*, *Allium* *Eichomia*, and *Oenothera*) that the stomatal movements were considerably more pronounced than would be expected from Lloyd's measurements. Other authors have found in their experiments on transpiration that

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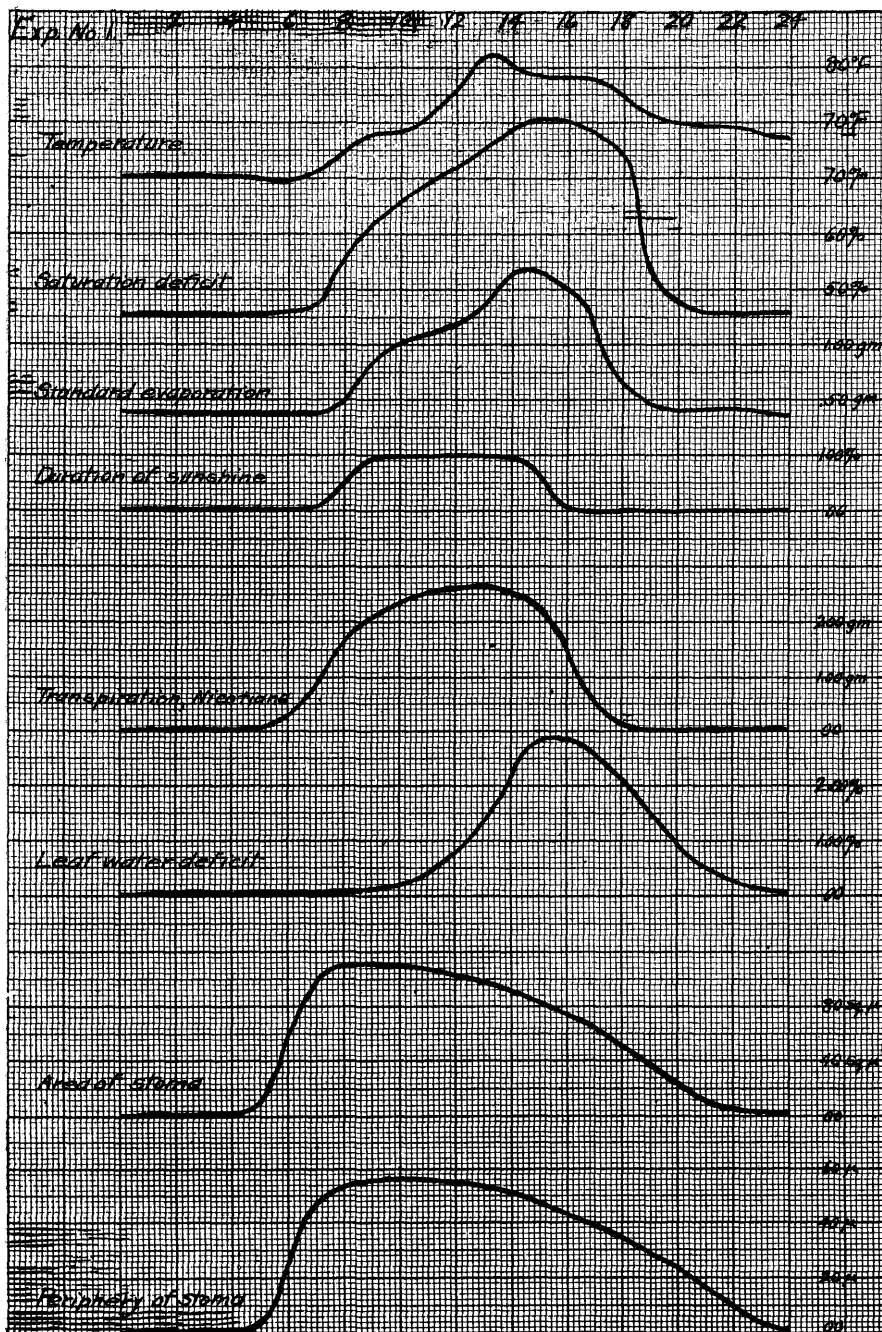


Fig. 1. Experiment 1.

many plants show a rhythm in the transpiration rates in darkness and attribute it to stomatal movements. Curtis⁴ kept several plants under constant conditions in darkness and determined their hourly transpiration rates by weighing. He refers to the behavior of the transpiration rates as "a pronounced periodicity in the stomata." He made no direct observations on the stomata but concludes that this rhythm was due to their movements. Darwin⁵ found by the indirect method of determining transpiration with a hygrometer that there was a rhythm in the transpiration rates in darkness and concluded that it was due to stomatal movements. Lloyd² found in "the plant (*Fouquieria splendens*) which he used that it behaves qualitatively the same in total darkness as under normal conditions until an hour (usually near 6 A. M.) when, the normal stimulation being absent, the plant relapses to a low condition of activity characteristic of its darkness condition. For the lack of a real explanation we must refer this behavior to the category of induced rhythm."

Lloyd measured the stomatal openings on different plants but could find no evidence that there was an increase in their dimensions corresponding to this induced rhythm. He concludes that some other factor than stomatal activity in the plant must be found to explain the transpiration curve. Livingston and Brown⁶ found that in nine out of eleven plants which they investigated there was a decrease in the water content of the leaves beginning after sunrise and reaching a minimum a short time after the transpiration attains its maximum. This decrease was as much as 8% in some plants and did not produce wilting of the leaves. A decrease in water content of the mesophyll cells increases the resistance of the cells to water loss. Livingston⁷ shows that some factor in the plant is operating to reduce water loss even though environmental conditions have not reached their maximum.

This summary shows that considerable work has been done on the effects of different factors on the transpiration curve; that no single factor has been found to control the rate of transpiration from the plant, and that transpiration is a resultant of the interaction of several internal and external factors.

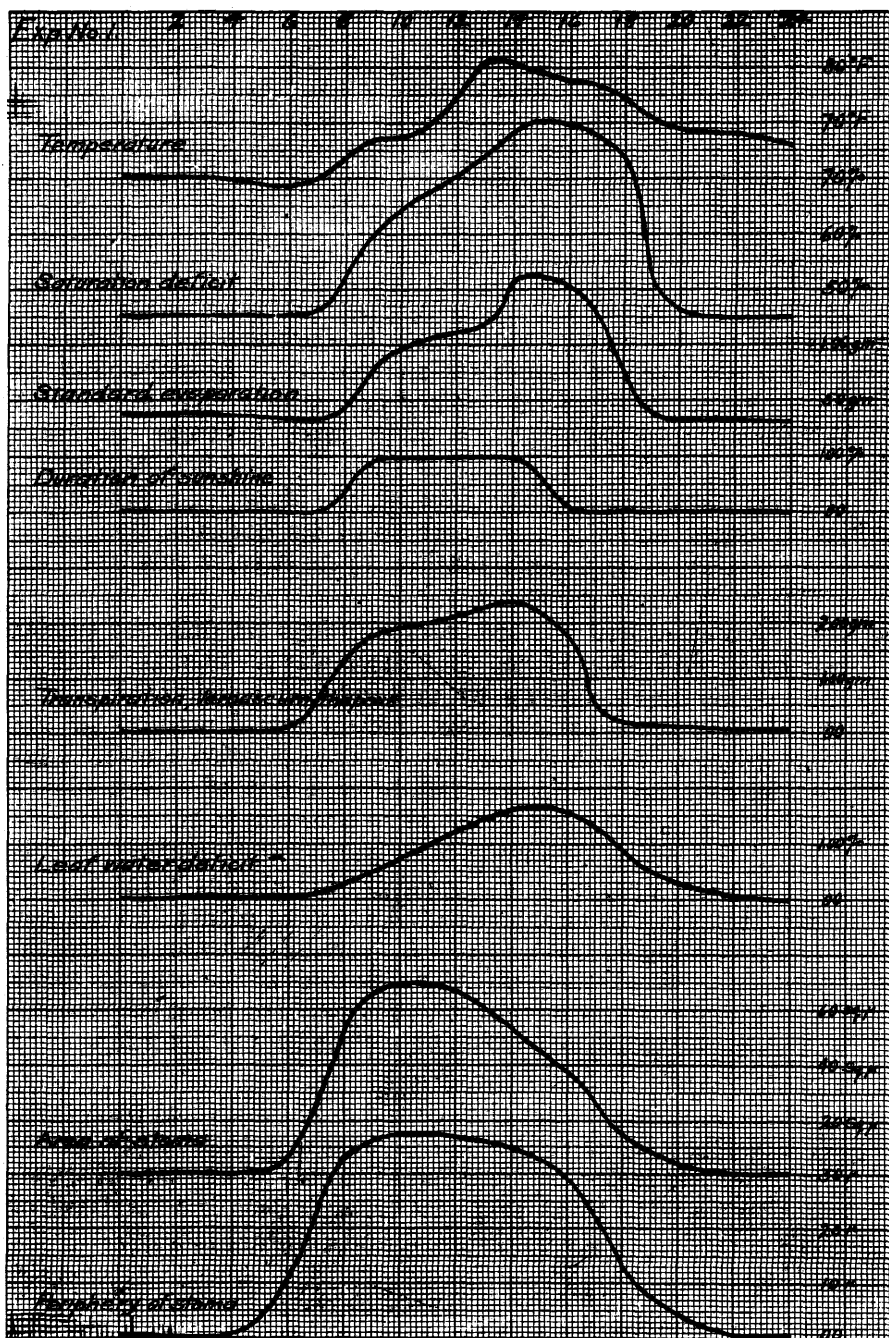


Fig. 2. Experiment 1.

EXPERIMENTAL METHODS.

The experiments reported in this paper were performed under the direction of Dr. E. N. Tanseau in the Plant Physiology Laboratory of the Ohio State University. The apparatus used in determining the water loss from the plants, which has been described by Transeau⁸, automatically records the loss of weight from sealed potted plants. In order to equalize light conditions in the greenhouse, the apparatus was mounted and operated on a rotating table. In the darkroom the apparatus was arranged on a laboratory table.

Records of the environmental factors influencing water loss from plants were obtained from instruments placed on the table with the other apparatus. The temperature and humidity of the air were recorded by a hygrothermograph, which was checked from time to time with a standard sling psychrometer. The evaporation rates were obtained from standardized porous cup atmometers. The record for the duration of sunshine was obtained from the U. S. Weather Bureau Station, Columbus, Ohio.

The water loss from the plants was measured as described by Transeau⁸ from potted, irrigated plants. Leaf areas of the plants were measured at the beginning and at the close of each experiment and all rates of water loss are given as the rate from one hundred square centimeters of leaf area, considering one surface of the leaf. Leaf water content was determined by weighing and drying leaves from plants treated exactly like those used in the determination of the water loss. The sizes of the pore openings of the stomata were measured from strips of epidermis fixed in absolute alcohol as suggested by Lloyd² except that the calculation of the dimensions was different.

The results of the experiments given in this paper are expressed by curves because they give a better representation of what took place in the experiment, besides occupying less space and allowing more results to be placed together for comparison. A standard method for plotting the curves was chosen and all curves, as nearly as possible, made in that way. The times in hours, beginning with 1 for 1 A. M., to 24 for 12 o'clock midnight, are used as abscissae, five millimeters equaling one hour. The rates of transpiration per hour, or variations per hour are used as ordinates.

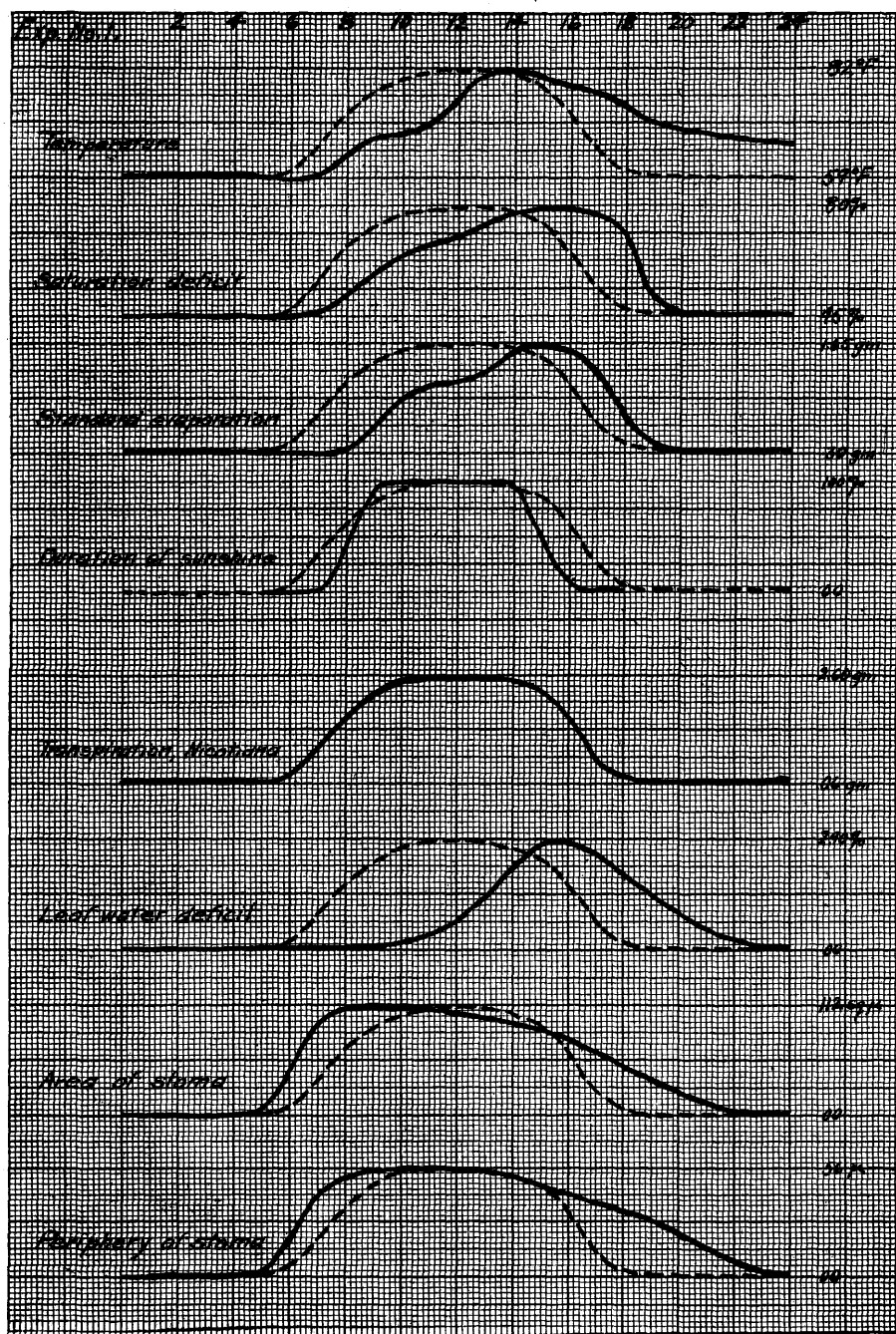


Fig. 3. Experiment 1.

The temperature is expressed in degrees Fahrenheit, and the humidity, reduced to saturation deficit ($100\% - \%$ of humidity) is expressed in percentages. The evaporation is given as the rate in grams per hour of water loss from the Standard Cup, calculated from the coefficients furnished by the Plant World Company. Duration of sunshine is expressed in per cents per hour of possible sunshine. Transpiration is given as the rate in grams per hour of water loss from one hundred square centimeters of leaf area. Leaf water contents are reduced to leaf water deficits by subtracting the figures for each from the maximum water content during the night, and are expressed in percentages. The areas of the stomatal pores and the peripheries of the stomatal pores are expressed in square microns and microns respectively.

EXPERIMENTS.

In Experiment 1 the leaf water contents and stomatal movements of tobacco and mullein were measured, together with transpiration and the several environmental factors. These make possible a comparison of the effects of the various factors.

Five mullein (*Verbascum thapsus*) and five tobacco (*Nicotiana* sp.) plants were used in this experiment, one of each in determining the transpiration, and the other four in estimating the leaf water content and stomatal measurements. The plants were all sealed and irrigated, and were given special care for a week before they were used, and during the experiment they were exposed to the same conditions.

The apparatus for determining the transpiration was automatic and needed very little attention, while the leaf water content and stomatal measurements required hourly observations. The method of making these observations was to strip small pieces of the lower epidermis from the leaf, put them at once in absolute alcohol, and then cut the leaf from the plant and place it in an air tight weighing bottle. The leaves were weighed at once and dried to constant weight and the water content calculated from the results. The pieces of epidermis were mounted on a slide in absolute alcohol and outlines of a representative number of pore openings drawn on paper with a camera lucida. Later these outlines were measured and reduced to their actual size in microns. A polar planimeter was

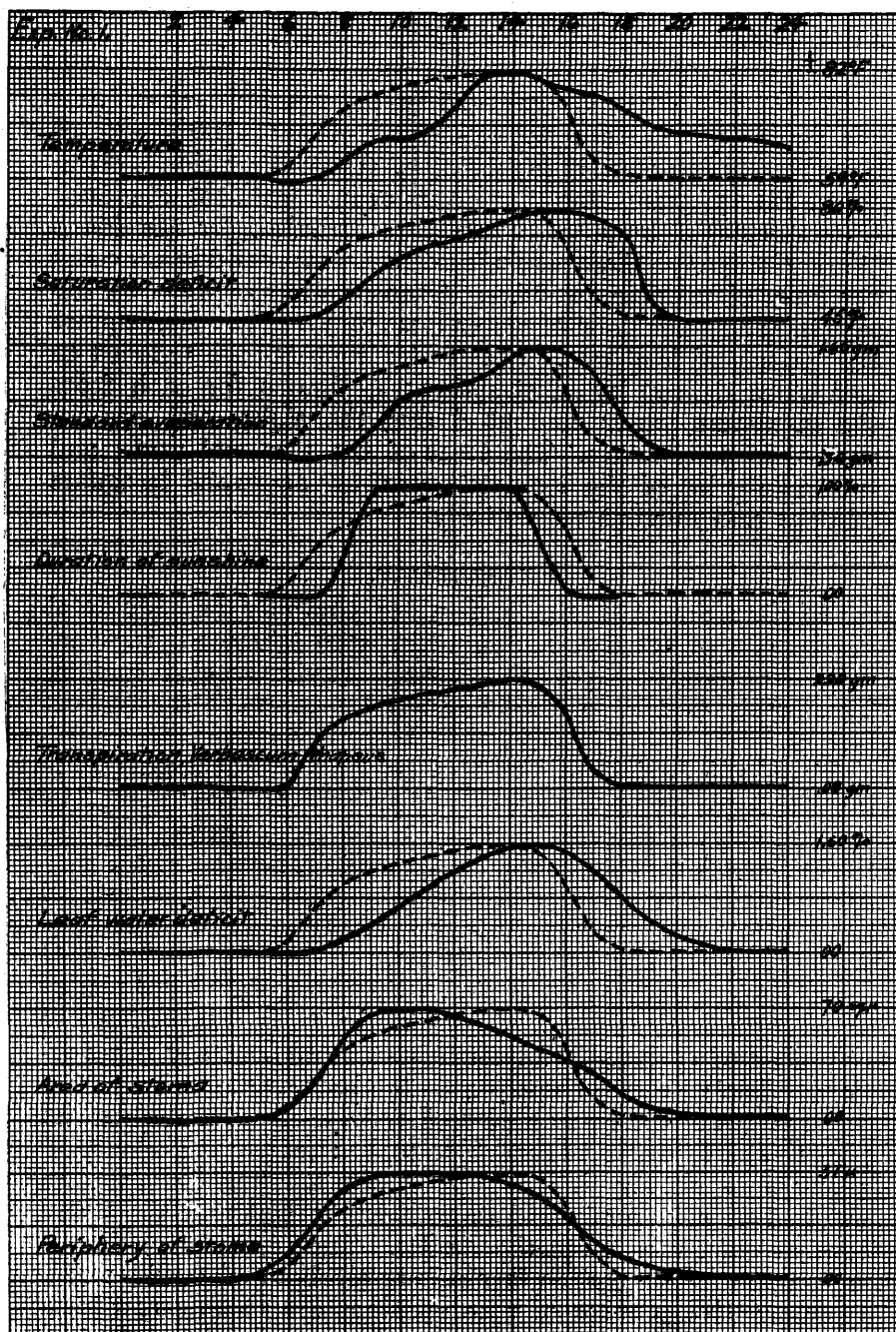


Fig. 4. Experiment 1.

used for measuring the areas of the openings and a small flexible centimeter rule for the peripheries.

In this experiment as well as in the preliminary work which was necessary to become familiar with the method, it was found that 85% or more of the stomata examined were closed at night, and as far as could be determined with the high power of the microscope this closure was complete and prevented gases from diffusing through them. In sunlight it was found that 90% to 96% were open. This difference is due, probably, to the fact that some of the stomata were fixed, or had ceased to open and close. Those that were found closed were regarded as zero in dimensions in calculating the area and periphery, and the results are expressed as an average of the number observed rather than the number found open. The results do not represent correctly the size of an average opening, because of their elliptical shape and the manner in which they close, but more nearly the average diffusion capacity of the stomatal area of the leaf. This method of observing and calculating the average size of the stomatal openings was followed in Experiment 1 and the results are given on pages 492 and 494.

These curves give the transpiration rates of mullein and tobacco, size of stomata, leaf water deficit and other factors of the environment. An inspection of these curves shows that the maximum temperature during the day occurs about the 13th hour and the maximum saturation deficit about the 15th hour. The maximum evaporation occurs about the 14th hour because it depends mainly upon the temperature and saturation deficit. The minimum of these factors occurs during the night.

The day was clear and quiet and there was full sunlight except for a short time, both in the morning and evening, when misty clouds occurred near the horizon. The transpiration curves began to rise about the sixth hour and rose rapidly until the ninth hour when the rise became more gradual until the maximum was reached at the 13th hour in tobacco and the 14th hour in mullein. This occurrence of the maximum rate of water loss before the maximum evaporation shows that some factor in the plant is operating to reduce water loss. The minimum of the transpiration curve occurs during the night. The leaf-water deficit began to rise gradually about the eighth hour and continued until a maximum was reached about the 15th or 16th hour when there was a decline to the usual amount by the

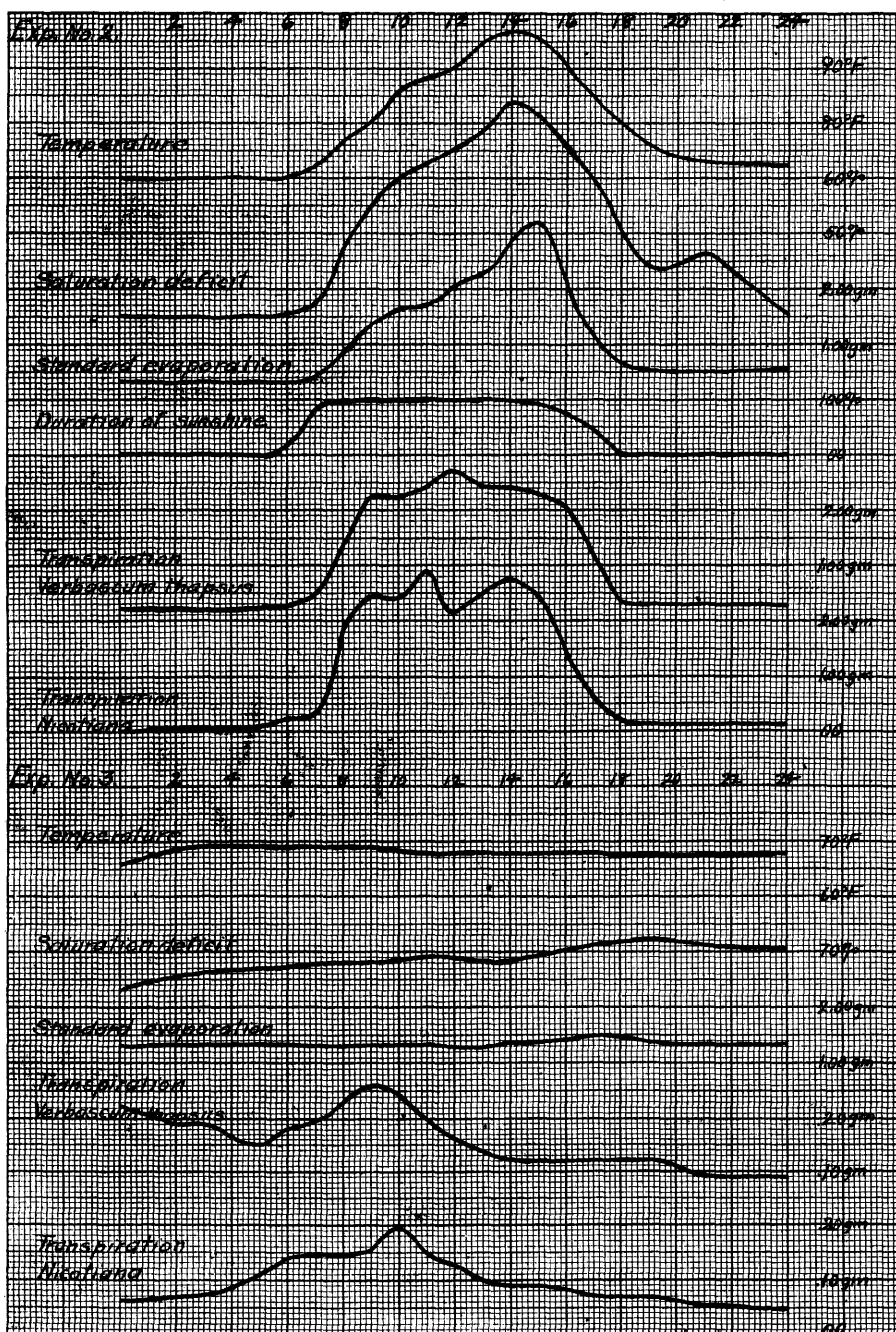


Fig. 5. Experiments 2 and 3.

22nd hour. The curves for the size of the stomata rose rapidly at the fifth hour, or sunrise, the hourly increment diminishing slowly until a maximum was reached at the 10th hour. From the maximum there was a gradual decrease until their night values were reached between the 20th and 22nd hour. It is apparent from the occurrence of the maxima of the different factors and the rates of water loss that no single factor controls the water loss from the plants.

A comparison of these curves does not give a true representation of their relations to each other because different scales of ordinates are taken in plotting them. For comparing curves of this kind the variation between the maximum and minimum values should be reduced to the same scale in all cases. The same curves are shown on pages 496 and 498 with the maximum and minimum values reduced to the same scale, and with the transpiration curve (dotted line) superimposed on each of the other curves. The maximum and minimum values of each curve are indicated on the base lines. A comparison of these maximum and minimum values shows that a change of 23° F. and 35% in saturation deficit of the air produced a change from 0.30 to 1.65 grams per hour in evaporation, *i. e.* increased it about five times. Under the same conditions of temperature and saturation deficit the transpiration rates showed a change from 0.08 to 2.20 grams per hour in mullein *i. e.* an increase of about 25 times and 0.06 to 2.60 grams per hour in tobacco *i. e.* an increase of about 40 times. It is obvious that so great a variation in the rate of water loss when compared with evaporation must be due to some physiological change within the plant.

An increase in leaf water deficit decreases transpiration by increasing the resistance of the mesophyll cells to water loss and as the maximum occurs a little later than the maximum water loss it operates to decrease the difference between the maximum and minimum rates of water loss. The stomatal openings are never used to their full diffusion capacity under ordinary conditions so the increase in their dimensions accounts for only the general difference between the day and the night rates of water loss. The rate of water loss from the leaf when the stomata are open is determined by the diffusion gradient (Renner,⁹), *i. e.*, the difference between the saturation deficit of the intercellular spaces of the leaf and the saturation deficit of the air.

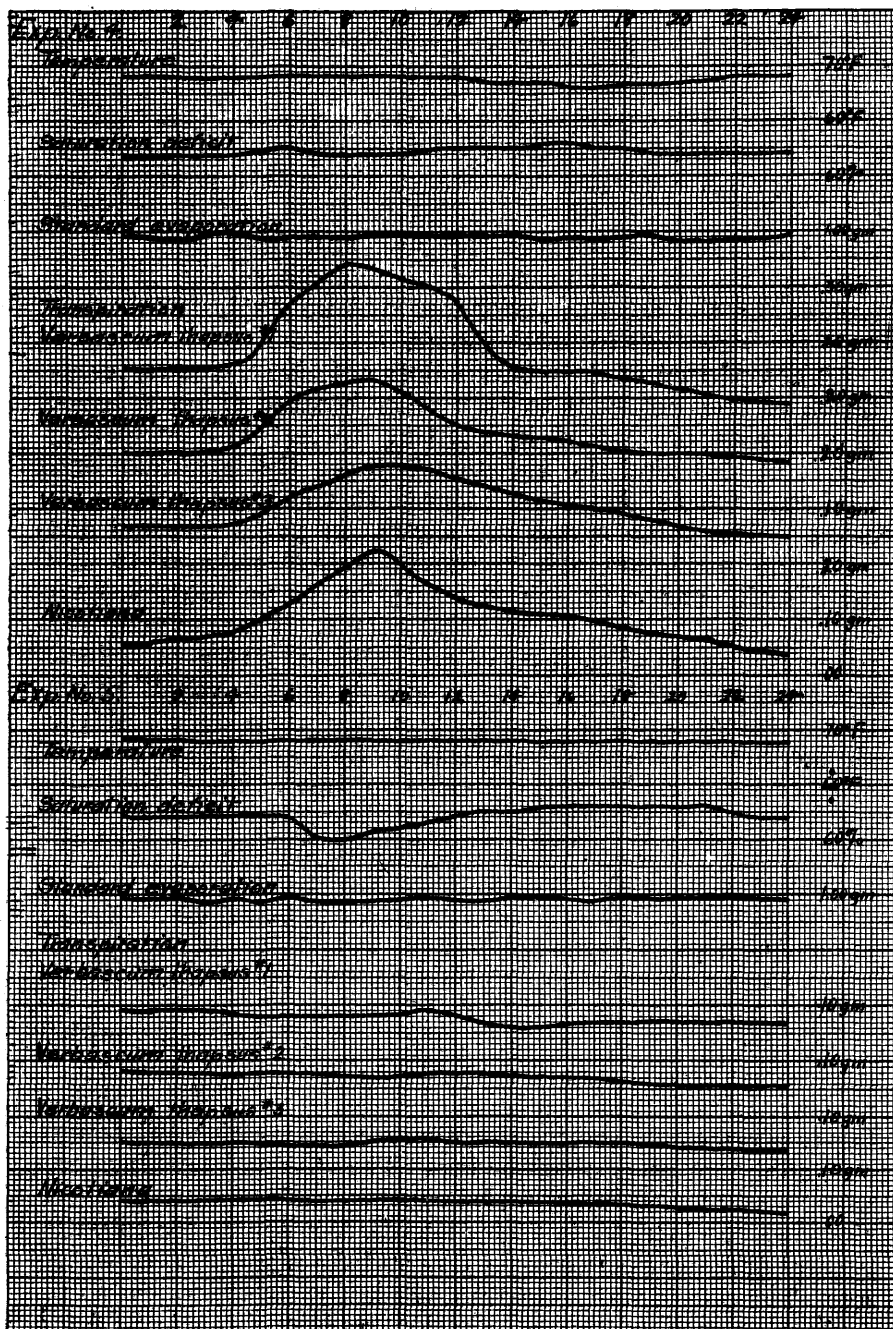


Fig. 6. Experiments 4 and 5.

The following conclusions concerning the limiting factors which control water loss from mullein and tobacco are based on Experiment 1. The diagrams on page 508 show the transpiration curves and the operation of these limiting factors. At night transpiration is limited to cuticular evaporation because the stomata are closed and the rate of water loss is determined by the temperature of the leaf and the humidity of the air, which were in these experiments nearly constant during the night. The process of water loss at night is simply and easily explained, but in the daytime the stomata open and there are changes in temperature and humidity due to sunlight which make the explanation of the process more difficult. The rate of water loss in the daytime is affected by the diffusion gradient of the stomatal pores although the cuticular transpiration still continues and is slightly increased by the rise in temperature and saturation deficit of the air. The increase in the rate of water loss during the morning hours is brought about by the increase in size of the stomatal pores, beginning about sunrise and the probable increase in diffusion gradient through the stomatal pores, resulting from the increased saturation deficit of the air. At about noon, there are two factors, leaf water deficit and decrease in stomatal pores, operating to diminish water loss from the leaf, and only one factor, diffusion gradient, tending to increase it. This results in a rounded curve and finally a steady decrease. The diffusion gradient becomes an additional factor tending to decrease water loss after the saturation deficit reaches a maximum and there are then three factors all operating in the same direction which cause the rate to quickly decline to the night level. Thus the water loss is reduced to the night rate, even though the stomata are not fully closed.

The stomata open normally every morning in sunlight and the normal transpiration curve shows a sudden rise at that time. The fact that the stomata open slightly but fail to open fully or to remain open if the plant is in continuous darkness in the morning has been inferred by Curtis and Darwin, who found a rhythm in the transpiration curve in darkness and concluded that it was caused by stomatal movements, but Lloyd, although he showed that an induced rhythm of the transpiration curve occurs in ocotillo, could find no corresponding increase in the size of the stomata. A number of experiments were performed which show this rhythm of the transpiration curve in mullein and tobacco.

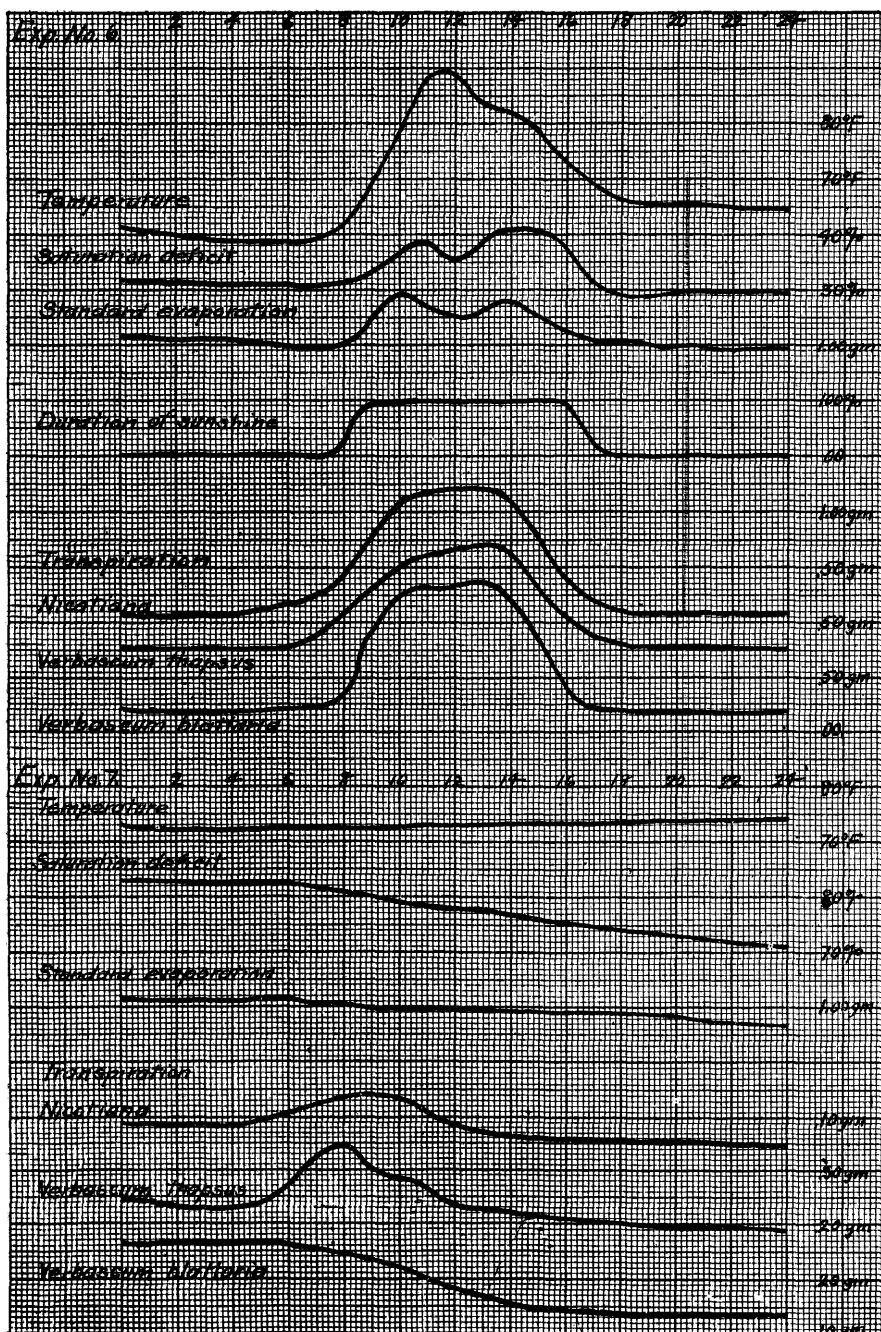


Fig. 7. Experiments 6 and 7.

Experiment 2: The curves for this experiment show the daily variation in transpiration of mullein and tobacco under ordinary conditions, with the environmental factors which influence water loss from plants. The transpiration rates show a rapid rise shortly after sunrise with high rates of water loss as compared with the night rates during the period of sunlight.

Experiment 3: This experiment shows the transpiration curves for the same two plants in a dark-room. The temperature, saturation deficit of the air, and evaporation were constant in the dark-room. But in spite of these constant environmental conditions the transpiration rates show a rise and fall or rhythm during the day. This rise in the rate of water loss begins about the time the stomata usually open or about the fourth or fifth hour and reaches a maximum about the ninth or tenth hour when there is a gradual decrease to the usual night rate by the middle of the day.

Experiment 4: In order to show that this rhythm was not characteristic of a single plant, different plants were put in the darkroom and their transpiration curves obtained. The curves from three mullein plants and one tobacco plant show this rhythm under conditions of constant temperature, humidity, and evaporation and in total darkness, which began, reached a maximum, and declined to the usual night rate similar to the curves of Experiment 3.

Experiment 5: The plants used in Experiment 4 were left in the dark-room and the curves for the second day under constant environmental conditions were obtained. There are no indications of a rhythmic rise and fall in the transpiration rates in this experiment.

Experiment 6: These curves show the hourly rates of transpiration and the related environmental factors in the greenhouse for three different plants, mullein, tobacco, and moth mullein (*Verbascum blattaria*). These plants have similar transpiration curves under these conditions. These mullein and tobacco plants were not used in any of the preceding experiments.

Experiment 7: These curves are from the same three plants that were used in Experiment 6, but they were obtained under constant environmental conditions in the dark-room. The curves for the rate of water loss from mullein and tobacco show

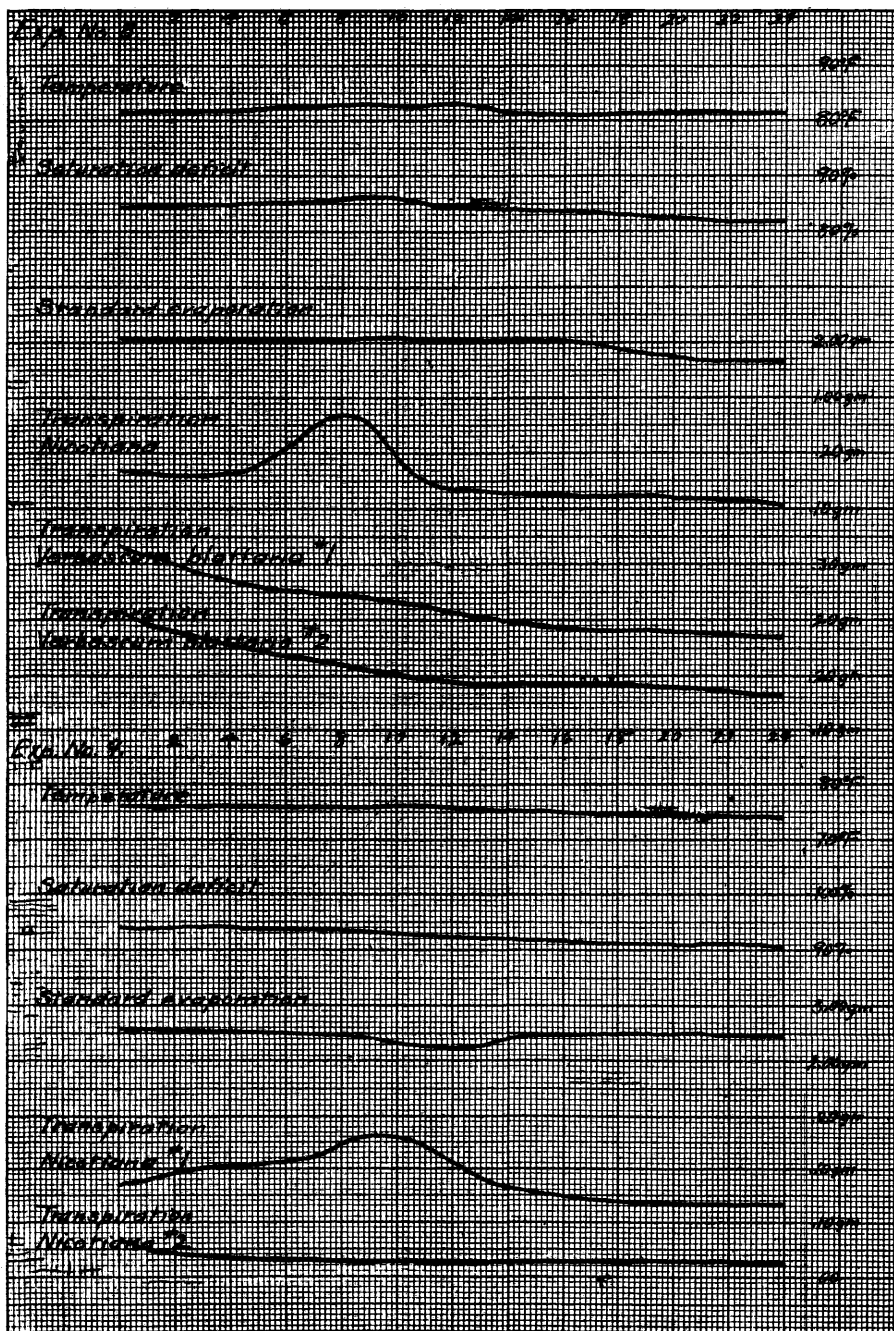


Fig. 8. Experiments 8 and 9.

rhythmic rise and fall similar to those from other plants the first day in the dark-room, but there are no indications of this rhythm in the rate of water loss from the moth mullein.

Experiment 8: To find out if the absence of a rhythm in the transpiration curve of moth mullein was a characteristic of that species or simply of an individual of the species, two more moth mullein plants and a tobacco plant were placed in the dark-room and their transpiration curves determined. But as in Experiment 7 under constant environmental conditions both of the moth mullein plants failed to indicate any rhythmic rise and fall in the rate of water loss, which showed a gradual decrease throughout the experiment.

Experiment 9: In this experiment the transpiration curves from two tobacco plants were obtained by placing *Nicotiana* No. 1 in the dark-room after sundown and *Nicotiana* No. 2 about noon of the preceding day. There was the usual rhythm in the transpiration curve in *Nicotiana* No. 1, but *Nicotiana* No. 2 showed no indications of this rhythm.

SUMMARY OF RESULTS.

The experiments given in this paper show that the differences between the night and day rates of water loss from mullein and tobacco are largely due to the differences in diffusion through the stomatal pores. Transpiration from the leaves at night is entirely cuticular and its rate is controlled by the temperature and humidity of the air when the stomata are closed. But the day rate is controlled by a number of factors operating to increase or decrease it. When the stomata open the diffusion gradient, or the difference between saturation deficit of the intercellular spaces and the atmospheric saturation deficit, causes a sudden rise in the rate of water loss. This rate continues until the leaf water deficit probably decreases the diffusion gradient by increasing the resistance of the leaf to water loss. After the leaf water deficit reaches a certain point there are two factors, leaf water deficit and decreasing stomatal pores, operating to reduce water loss. There is only one factor, diffusion gradient, tending to increase it. This results in a rounded curve as shown in figure 9. After the saturation deficit reaches a maximum all three factors operate to decrease the rate of water loss. This results in a rapid decline in the rate,

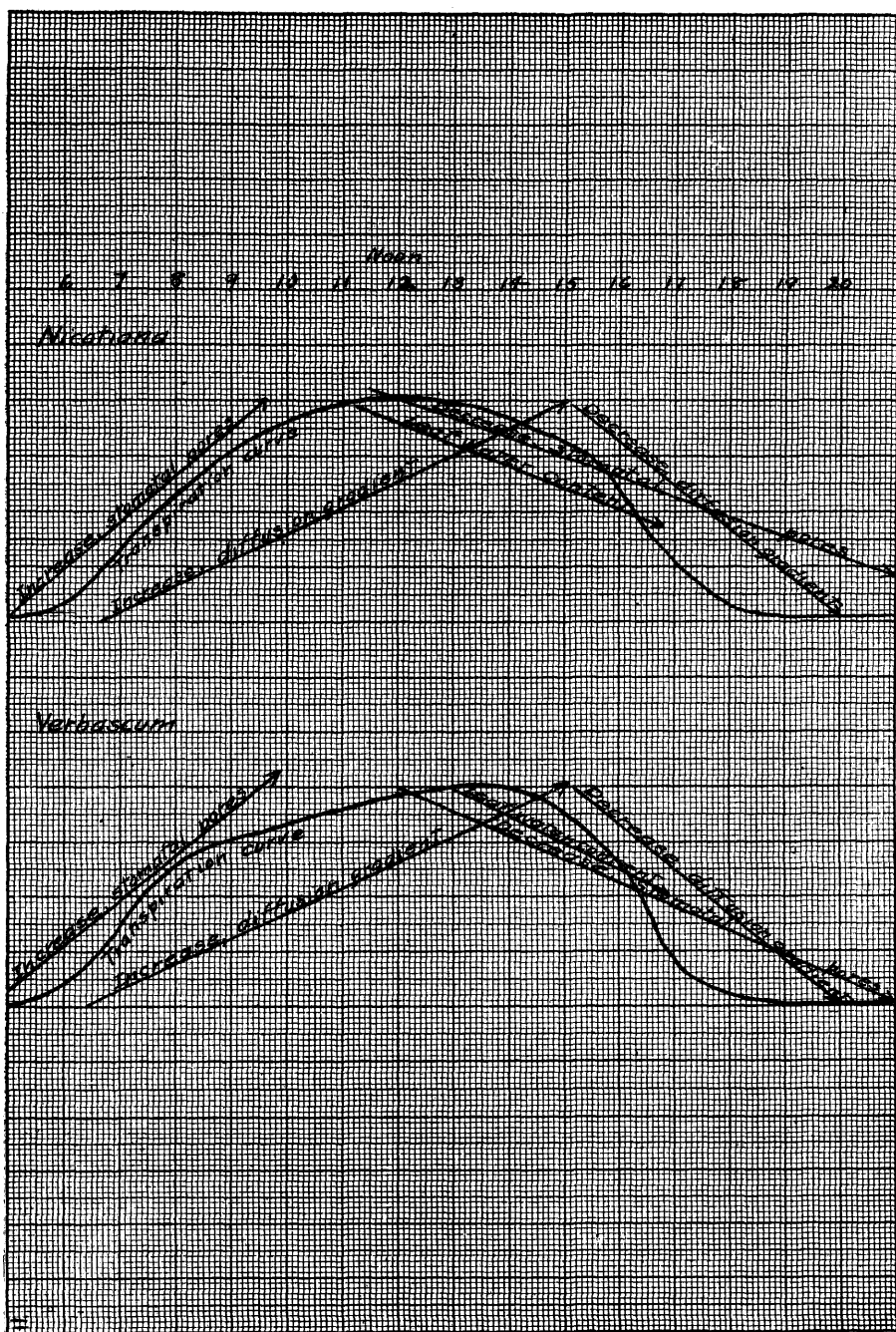


Fig. 9. Diagrams showing factors which modify the rate of transpiration.

even before the stomata are fully closed. The decreasing leaf water deficit has no effect after the stomata are closed.

Tobacco and mullein show a rhythm in the transpiration curve in total darkness when preceded by a day of normal light conditions, while moth mullein under the same conditions does not show this rhythm. This rhythm is expressed as a rise in the rate of water loss at about the time the stomata usually open in sunlight with a maximum about the middle of the forenoon and a decrease to the usual night rate by noon. This takes place in total darkness under constant environmental conditions. The rhythm in tobacco and mullein does not show up on the second day in the dark-room, and in tobacco which is placed in the dark-room about noon of the preceding day there is no rhythm in the transpiration curve. The moth mullein does not show this rhythm under the same conditions. It seems therefore, that certain plants have this characteristic rhythm while others do not. The cause of the rhythm is most likely stomatal activity but because of the large errors in measuring these movements as compared with the small movement necessary to produce the slight increase in transpiration it has not been found possible to verify it.

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